The African Organisation for Standardisation

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ARS 844 (2012) (English): Determination of cyanogens in cassava products

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Cassava and cassava products — Determination of total cyanogens — Enzymatic assay method

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Introduction

Cassava *(Manihot esculenta* Crantz) is one of the staple food crops. A major drawback of cassava utilization is its potential toxicity due to the presence of endogenous cyanogenic glucosides.

Processing of cassava should reduce the cyanogenic glucosides to an acceptable level. This test method therefore, is intended to be used for determination of the cyanogen content in cassava and its different products as a measure to safeguard the health of the consumers.

Cassava and cassava products — Determination of total cyanogens — Enzymatic assay method

1 Scope

This African Standard specifies a method for the determination of total cyanogens in cassava and cassava products.

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

No normative references have been cited in this standard.

3 Principle

Linamerase hydrolyses cyanogenic glucoside compounds of cassava extract in orthophosphoric acid medium to produce cyanohydrins which rapidly decompose to cyanide ion in alkali (NaOH), followed by addition of excess pH 6 buffer and chloramine-T to produce a purple solution which is measured spectrophotometrically

4 Reagents

4.1 Linamarin, stock solution (5 mmol) made by dissolving 31.0 mg of Linamarin in 25 ml buffer, pH 6.

NOTE Weighing should be done quickly as the compound is hygroscopic.

4.2 Linamarase, stock solution made by dissolving linamerase enzyme in a 0.1 M sodium phosphate buffer to obtain a 2.5 µg linamarin in 0.1 M aliquot

4.3 Isonicotic acid

4.4 1,3-dimethyl barbituric acid

4.5 Acetone cyanohydrin (99 %), stock (5 mmol) made by first dissolving 570 µl (0.532 g) in 25ml of 0.1 M orthophosphoric acid, and from this solution, making 1.00 ml up to 50 ml with 0.1 M orthophosphoric acid.

Because of the poor performance of the pure compound in a pipette, the added quantity should also be weighed for accurate calculation of concentration. The refrigerated stocks can be stored for several weeks to months.

4.6 Potassium cyanide (KCN), stock (5 mmol) made by dissolving 163 mg dry and pure KCN in 500 ml 0.2 M NaOH. KCN should be dried for at least 12 h over concentrated H_2SO_4.

Standard solutions of 80 µmol and 320 µmol are made from the stock solutions by making 1.60 ml of them up to, respectively 100 ml and 25 ml with 0.1 M orthophosphoric acid. This results in 8 nmol/tube and 32 nmol/tube and absorbance of about 0.220 absorbance unit (AU) and 0.880 AU depending on the spectrophotometer. KCN standards are made just prior to analysis and applied as 0.1 ml to 0.5 ml orthophosphoric acid and 3.4 ml buffer, pH 6 or as 0.1 ml to 3.9 ml buffer, pH 4.

4.7 Chloramine T solution.

4.8 Colour reagent, pure NaOH (3.7 g) is dissolved in 200 ml distilled water. 7 g of 1,3-dimethyl barbituric acid and 5.7 g isonicotinic acid are dissolved in this alkaline solution by extensive stirring. The pH is adjusted between 7 and 8 with 1M HCI or NaOH. This reagent can be kept for at least 12 days at room temperature.

NOTE Stock solutions and reagents should be of analytical grade.

5 Apparatus

Ordinary laboratory apparatus, and a spectrophotometer

6 Procedure

6.1 Sample preparation

Cut fresh cassava root or moist products into 1 cm cubes and randomly take 50 g cassava cubes and homogenize in 250 ml refrigerated 0.1M orthophosphoric acid in a blender for 15 s at low speed, followed by 60 s at high speed, 60 s of rest and 60 s at full speed again.

For flour 4 g is swirled gently in 25 ml of refrigerated extraction medium in a 50 ml closed container for 5 min.

Extracts are best analyzed immediately, but if not, they should be stored refrigerated or frozen for up to two months.

6.2 Analytical assay

6.2.1 Cyanogenic potential.

0.1 ml extract is added to 0.4 ml buffer, pH 7, in a test tube, followed by addition of 0.1 ml linamarase solution. After 15 min incubation at 30 °C, 0.6 ml NaOH (0.2 M) is added, followed after 5 min, by 2.8 ml buffer, pH 6.Coloration is as described in 6.3.

6.2.2 Non glucosidic cyanogens

0.1 ml extract is added to 0.6 ml of NaOH (0.2 M). After 2 min, 3.3 ml buffer, pH 6 is added, followed by coloration (6.3).

6.2.3 Free cyanide (HCN)

0.6 ml extract is diluted with 3.4 ml buffer, pH 6 and assayed by colorimetry (6.3).

NOTE Cyanogens are assayed in duplicate and all assays are carried out in glass-stoppered test tubes. The contents of the tubes are mixed after each addition.

6.3 Colorimetric procedure

0.1 ml Chloramine-T reagent (2 % w/v) is added to the 4 ml buffered extract in the test tubes and mixed. After 5 min, 0.6 ml of color reagent is added and mixed. The absorbance at 605 nm is measured after 10 min. Reagent blanks are run for each analysis. In a digital single beam spectrophotometer, the absorbance can be measured between 0.050 AU and 2.000 AU (Absorbance Unit). In less sophisticated analog equipment, absorbance can be measured between 0.100 AU and 1.200 AU with acceptable accuracy.

6.4 Calculation of cyanogen content

Cyanogen levels are calculated in mg HCN equivalent per kilogram sample on dry weight basis (mg HCN equivalent kg^{-1}, DWB) as follows:

$$\text{HCN equivalent} = \frac{0.027\,\chi\left[v+\frac{sm}{100}\right]}{ds\left[1-\frac{m}{100}\right]}$$

where,

s is the sample weight in grams (g);

v is the volume, in millilitres, of the extraction medium;

d is the volume, in millilitres, of extract assayed; and

m is the moisture content, %.

Dry 5 g to 10 g sample (to constant weight), at 105 °C ± 2 °C (oven) or 70 °C in a vacuum oven and calculate the moisture content (%).

χ is the quantity of cyanogens (nmol) in the tube, calculated from calibration curve as follows:

$$\chi = \frac{\left(A_{605} - w\right)}{\text{Slope}}$$

where,

A_{605} is the absorbance measured at 605 nm. Both slope and intercept "w" are derived by linear regression of the calibration points by means of a calculator. For very low values of "w" (<0.005) and higher values of A_{605} (>0.200) the intercept can be neglected.

7 Test report

The test report shall show the method used and the result obtained.

It shall also mention any operating conditions not specified in this standard, or regarded as optional, as well as any circumstances that may have influenced the result.

The report shall include all details required for the complete identification of the sample analyzed.

Annex A
(normative)

Determination of moisture content

A.1 Procedure

Weigh accurately 10 g of the material in a suitable moisture dish previously dried in an electric oven and weighed. Place the dish in an electric oven maintained at 105 °C ± 1 °C for 5 h. Cool the dish in a desiccator and weigh with the lid on. Repeat the process of heating, cooling and weighing at half-hour intervals until the loss in weight between two successive weighings is less than 1 mg. Record the lowest weight obtained.

A.2 Calculation and expression of results

$$\text{Moisture, percent by mass} = \frac{(M_1 - M_2)\,x\,100}{M_1 - M_3}$$

where,

M_1 is the mass, in grams, of the dish and sample before drying;

M_2 is the mass, in grams, of the dish and sample after drying;

M_3 is the mass, in grams, of the dish only.

Annex B
(informative)

Determination of free fatty acids

B.1 Apparatus

Soxhlet fat extraction apparatus

B.2 Reagents

B.2.1 Petroleum ether, distilling below 65 °C, or ethyl ether

B.2.2 Alcohol potassium hydroxide, 0.1 N (use absolute or alcohol denatured with methanol, [MeOH])

B.2.3 Alcohol-ether mixture, equal volumes of 96 % alcohol and ethyl ether

B.2.4 Phenolphthalein solution, 1 % in alcohol or alcohol denatured with methanol (MeOH)
Add 0.3 ml per 100 ml mixture of alcohol-ether and add alcoholic KOH solution to a faint pink.

B.3 Procedure

Extract 10.00 g ± 0.01 g of the sample taken in a thimble with petroleum ether for about 4 h in a Soxhlet extraction apparatus. Completely evaporate the solvent from the extraction flask (weighed previously) on a steam bath, cool and weigh the extraction flask with the residue. Dissolve the residue in the extraction flask with the 50 ml of the alcohol-ether phenolphthalein solution. Titrate the dissolved extract, with standard potassium hydroxide solution, to a faint pink colour, which persists for 10 s. If emulsion is formed during titration, dispel by adding a second 50 ml portion of the alcohol-ether phenolphthalein solution.

Make a blank titration on 50 ml of the alcohol-ether phenolphthalein solution and subtract this value from the titration value of the sample. If the additional 50 ml portion of the alcohol-ether phenolphthalein solution is added, double the blank titration.

B.4 Calculation

Calculate the acid value from the following formula:

$$\text{Acid value (as oleic acid)} = \frac{56.1VN}{M}$$

where,

V is the volume, in millilitres, of standard potassium hydroxide solution used;
N is the normality of standard potassium hydroxide solution; and
M is the mass, in grams, of the material taken for the test.

Bibliography

EAS 744:2010, *Cassava and cassava products — Determination of total cyanogens — Enzymatic assay method*